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Effect of Asbestos on the Metabolism of Vasoactive Substances in Isolated Perfused Guinea Pig Lungs

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The measurement of pulmonary metabolism of three vasoactive substances and quantitative assessment of changes in lung morphology were performed in a long-term study of asbestos-exposed guinea pigs. Animals received intratracheal injections of a single dose of a sterile suspension of Canadian chrysotile B (5 mg), while control animals received only saline. Six months after the treatment, the guinea pigs were sacrificed, the lungs removed, perfused via the pulmonary artery and the metabolism of vasoactive substances was assessed (*in vitro*) in a cascade superfusion system. At the end of the experiments, the lungs were fixed in a glutaraldehyde solution for microscopic examination. The tissue response consisted of both inflammatory reaction of terminal and respiratory bronchioles and diffuse alveolar septal infiltration with interstitial fibrosis. The reaction was characterized at six months by a progressive bronchiolitis obliterans with fibroblastic proliferation and collagen formation. The development of the disease did not cause significant changes in the metabolism of acetylcholine and bradykinin. However, the metabolism of prostaglandin E_2 decreased with the appearance of the bronchiolitis obliterans. Our results showed that asbestos exposure may produce early biochemical changes resulting in altered lung metabolism of vasoactive substances; these modifications could contribute to the pathogenesis of asbestosis.

Introduction

Lungs occupy a strategic position in the body. This organ establishes the principal link between the external environment and the organism. Besides its ability to perform vital gas exchanges, the respiratory system is also designed to protect the body against external intruders such as infectious agents or foreign materials. These important defense functions depend on the combined and integrated effects of several mechanisms, namely the action of the pulmonary macrophages, the mucociliary transport and the immune system (*1*).

On the other side of this biological barrier, the lungs are also involved in the hormonal control of blood circulation. The vast surface covered by the pulmonary vascular bed and especially the numerous enzymatic activities found on and in the capillary endothelial cells give to the respiratory system the capabilities of activating and inactivating a large series of vasoactive substances (*2*, *3*). Disturbance of these basic physiological processes with physical or chemical means could have dramatic consequences on homeostasis.

The aim of the following experiments was to investigate the effects of asbestos exposure on the nonrespiratory functions of the lungs. The fate of bradykinin, acetylcholine and prostaglandin E_2 in the pulmonary circulation of guinea pigs following asbestos exposure was studied.

Materials and Methods

Experimental Asbestosis

Guinea pigs of either sex weighing between 350 and 450 g were given a single intratracheal injection of 0.25 mL of a 20 mg/mL sterile suspension of Canadian chrysotile B (UICC). The total amount of asbestos fibers introduced into the lungs was 5 mg per animal. They were housed four to six per cage and kept under constant environmental conditions until use. Water and food were given *ad libitum*.

Isolated Lung Preparation and Bioassay

Six months after the administration of the asbestos suspension, the animals were sacrificed by cervi-

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cal dislocation. The thorax was opened and heparin (500 IU) was given intracardially. The lungs were removed and perfused via the pulmonary artery with oxygenated Krebs solution at 37°C essentially as described previously by Alabaster and Bakhle (*4*). The perfusion rate was maintained at 5 mL/min, and the effluent from the lungs superfused over selected isolated smooth muscles preparations in a cascade perfusion system (*5*). The following assay tissues were used: the rat stomach strip (*6*) for prostaglandin E_2 (PGE_2), the guinea pig ileum (*7*) for bradykinin (BK) and the external muscular layer of the guinea pig ileum (*8*) for acetylcholine (Ach). The specificity and stability of the tissular response were insured by the infusion of a mixture of inhibitors containing: methysergide (M), 0.5 μg/mL; propranolol (P), 3.0 μg/mL; phentolamine (Ph), 1.0 μg/mL; atropine (A), 0.5 μg/ml; and diphenhydramine (D), 1.0 μg/mL for the bioassay of PGE_2 and BK and by the infusion of M.P.Ph.D. for the bioassay of Ach. The perfusion solution also contained indomethacin (1.0 μg/mL) to prevent release of prostaglandins from the lung. Contractions of the assay tissues were recorded with isometric force displacement transducers (Grass FT03) attached to a multichannel Grass polygraph (Model 7D). The amount of BK, Ach and PGE_2 removed from the perfusion medium by the lungs was determined by comparing the contraction of the assay tissues to bolus injections given directly into the superfusing fluid with responses to bolus injection given through the pulmonary arterial cannula.

Histopathological Methods

At the end of each perfusion, the lungs were fixed by immersion in a phosphate-buffered 4% formaldehyde-1% glutaraldehyde solution for histological examination (*9*). Representative 5 μm paraffin sections were cut and stained with hematoxylin-eosin.

Drugs

Acetylcholine chloride and atropine sulfate were purchased from Sigma Chemical Co. The following drugs were supplied as generous gifts: prostaglandin E_2 (Dr. J. E. Pike of UpJohn Co.), bradykinin (Dr. D. Regoli, Dept. Pharmac., University of Sherbrooke), methysergide hydrogen maleate (Sandoz Ltd), propranolol hydrochloride (Dr. L. Smith, Ayerst), phentolamine hydrochloride (Ciba Lab.), diphenhydramine hydrochloride (Dr. E. McMullen of Parke, Davis & Co.) and indomethacin (Merck Frosst Lab.).

PGE_2, BK and Ach were dissolved in Krebs solution just before use. Suspension of Canadian chrysotile B (UICC) (20 mg/mL) were made in physiological saline and autoclaved before use.

Results

Morphological Effects of Asbestos on Guinea Pig Lungs

The histological appearance of the lungs of guinea pigs exposed to chrysotile asbestos is shown in Figures 1 and 2. In general, the morphological changes are representative of the early stage of development of the disease. Intratracheal injections produced nonuniform distribution of the asbestos fibers, and consequently the repartition of pulmonary lesions is slightly different from one animal to the other. After 6 months of exposure, some large fibers persisted in small airways but most of the smallest ones were found in the adjacent interstitial spaces and alveoli. Distal lung structures were more affected, and the most significant lesions were located in and around terminal bronchioles. They were characterized by three principal manifestations. The first and main feature observed was a granulomatous mainly peribronchiolar reaction which resulted in severe structural distortion of the terminal and respiratory bronchioles. As seen in Figure 1, the zone of reaction was sometimes so extensive as to also obliterate surrounding alveolar spaces. Asbestos bodies were present in great numbers in these areas but were not apparent with the type of staining used on Figure 1. A few sites of cuboidal metaplasia of some alveolar walls, always adjacent to damaged bronchioles, led to the apparition of an adenomatoid appearance. Finally, focal fibroblastic proliferation induced in peribronchiolar tissues distorted and obstructed small airways (Fig. 2).

These features of peribronchiolitis and bronchiolitis obliterans were seen in all animals exposed to chrysotile asbestos.

Metabolic Effects of Asbestos

The effect of exposure to asbestos dust on the pulmonary metabolism of bradykinin, acetylcholine and prostaglandin E_2 is shown in Figure 3. These recordings represent the results of typical experiments in which the three assay tissues were directly superfused with the effluent from perfused guinea pig lungs. Each one has been selected from different experiments. For each substance studied, bolus injections through the pulmonary artery were repeated with three different doses (in triplicate). Values of percent removal obtained with each of the substances represent the mean of nine consecutive injections.

As shown on Table 1, no changes in the inactivation of bradykinin were observed in treated lungs when compared to control animals. The lungs removed 88% of a bolus injection of 100 to 400 ng of

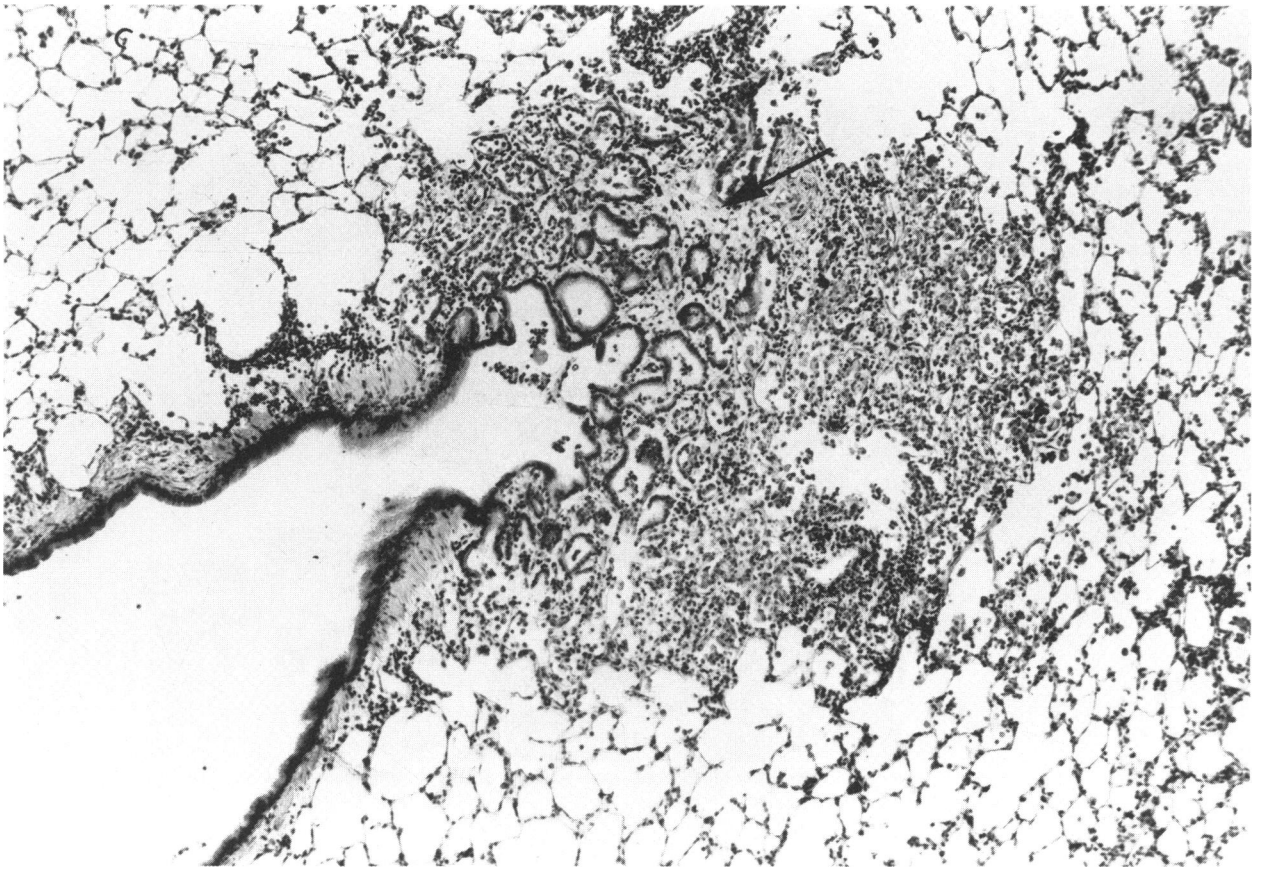

FIGURE 1. Guinea pig asbestosis. The photomicrograph reveals the peribronchiolar granulomatous reaction. Note the severe structural distortion of the respiratory bronchiole. Arrow points out an area of so called "adenomatoid" appearance formation.

Table 1. Removal of bradykinin (BK), acetylcholine (Ach) and prostaglandin E_2 (PGE_2) in isolated normal and asbestotic guinea pig lungs.

Drugs (and doses used, ng)	Removal, %[a]	
	Control	Asbestos (6 months)
BK (100-400)	87.9 ± 1.86 (30)	87.6 ± 1.96 (21)
Ach (100-1000)	82.4 ± 2.7 (11)	82.0 ± 2.8 (5)
PGE_2 (1-20)	83.0 ± 3.02 (18)	63.6 ± 5.2 (20)

[a]Results are expressed as mean ± SEM. The number of experiments is given in parentheses. For details, see "Materials and Methods" and Figure 3.

the peptide given through the pulmonary circulation. This high degree of inactivation was maintained at the same level after 6 months of chrysotile exposure.

The same phenomenon was observed with the cholinergic agonist. Mean values of 82% of lung removal (as measured by the changes of the height of the peaks) remained unchanged for doses from 100 to 1000 ng of Ach in controls and in treated animals. An average of 18% of the amount injected was detected in the lung perfusates (16 experiments).

In contrast to these results, the removal of prostaglandin E_2 was significantly altered ($p<0.005$) by asbestos exposure. From 83% in the control animals, the inactivation of PGE_2 decreased to 63% when the fatty acids were passed through lungs of exposed guinea pigs. This diminution in the metabolism was reproduced with doses of 1 to 20 ng of PGE_2. In other words, the effect of asbestos treatment causes two-fold increases in the amount of PGE_2 emerging from the lungs following a single bolus injection.

Discussion

Asbestosis is a chronic disease which requires several years to develop in humans. In experimental animals, it is not only possible to reproduce the characteristic tissue damages of asbestos exposure but mainly to accelerate their apparition by injecting the fibers directly into the trachea (*10*). Our results showed that intratracheal injections of asbestos to guinea pigs induced in a few months a fibroblastic proliferation with all the main features of the bronchiolitis obliterans. Although the distri-

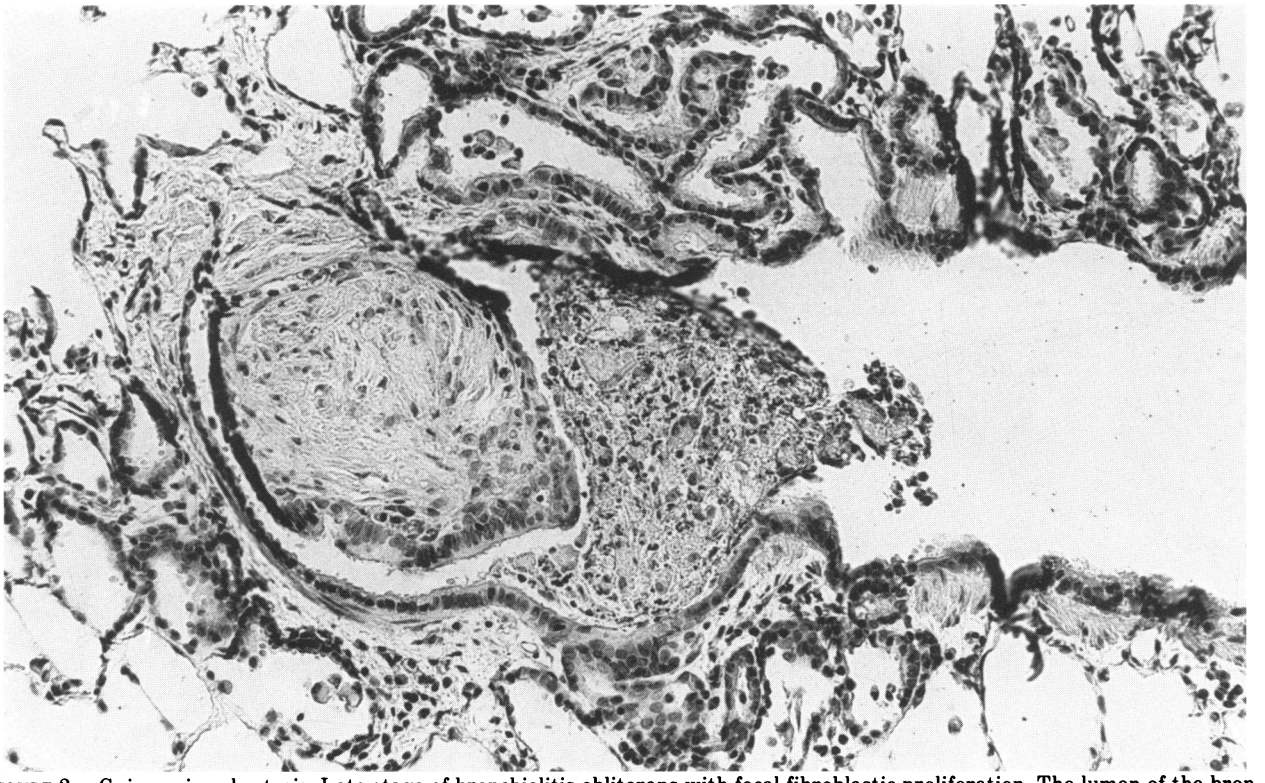

FIGURE 2. Guinea pig asbestosis. Late stage of bronchiolitis obliterans with focal fibroblastic proliferation. The lumen of the bronchiole is almost obliterated by the cellular invasion.

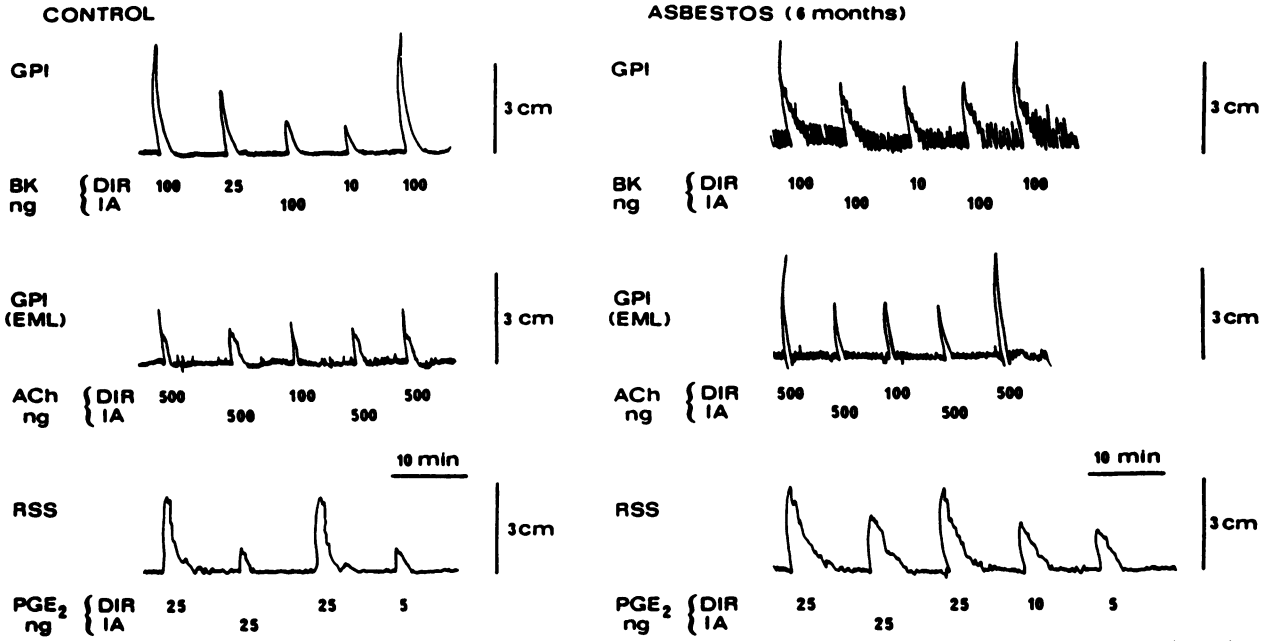

FIGURE 3. Typical recordings of the pulmonary inactivation of bradykinin (BK), acetylcholine (Ach) and prostaglandin E_2 (PGE_2) in control guinea pigs (left) and in animals which have received intratracheal injection of 5 mg of asbestos fibers 6 months before (right). The top record shows contractions of the guinea pig ileum (GPI) to BK. The middle one represents the contractions of the external muscular layer of guinea pig ileum (GPI/EML) to Ach, and the bottom one shows the response of the rat stomach strip (RSS) to PGE_2. For each substances, the doses have been injected either directly (DIR) over the superfused tissue or through the lung (IA) via the pulmonary artery.

bution and the degree of development of the disease was slightly variable from one animal to the other, the three histological manifestations, namely, the peribronchiolar reaction, the adenomatoid appearance of the tissue adjacent to the damaged bronchioles and the focal fibroblastic proliferation, were present in most animals. These morphological changes, consistent with the development of pulmonary fibrosis, confirmed the findings reported by several investigators with rats (*11*), hamsters (*12*) and guinea pigs (*13*, *14*).

During the development of experimental asbestosis in guinea pigs, the pulmonary metabolism of bradykinin and acetylcholine was not altered. The values remained, respectively, at 88% and 82% of metabolism for controls and for asbestos-treated animals. However, the metabolism of prostaglandin E_2 was significantly reduced by asbestos treatment. It fell from 83% in control animals to 63% in treated ones. Although a 20% decrease in pulmonary removal may appear of minor importance, it means that twice as much prostaglandin can escape lung metabolism (from 17% of the dose injected in controls to 37% in treated animals) and enter the systemic arterial circulation.

These findings are particularly interesting since they pointed out a marked difference among the mechanisms involved in the pulmonary metabolism of the three substances studied. PGE_2 is the only substance of the three which need to enter the cells to be metabolized. Bradykinin is destroyed by a converting enzyme located on the luminal surface of the pulmonary endothelial cells (*15*), whereas acetylcholine is believed to be inactivated by local cholinesterase (*16*). Prostaglandin inactivation in lungs depends on an active uptake into the cells and subsequent metabolism by intracellular enzymes. The activity of the 15-hydroxyprostaglandin dehydrogenase was found to be the rate-limiting step of PGs inactivation (*17*). Our findings suggest that the decrease observed in the pulmonary metabolism of PGE_2 could be due either to the inhibition of this enzyme or to inhibition of its transport into the cells. But how such an injury to the lung could interfere with the activity of this intracellular enzyme or with the membrane transport remains an open question. Further studies are needed to clarify the phenomenon.

In summary, our results have shown that intratracheal injections of asbestos fibers in guinea pigs produced a bronchiolitis obliterans with fibroblastic proliferation and collagen formation and altered the pulmonary inactivation of prostaglandin E_2 but not of bradykinin and acetylcholine. The physiological significance of these findings as well as the role of lung metabolism in homeostasis remains speculative.

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